the major histocompatibility complex (MHC). However, it remains unknown how this interaction is translated into intracellular signaling. Three different models of TCR triggering have been suggested which are based on aggregation, segregation or a conformational change of the TCR. Here we exploit a combination of super-resolution fluorescence microscopy and single-particle tracking to follow how the spatial distribution of proteins essential to T-cell activation like the TCR, CD45 and Lck changes at the interface of live T-cells and various surfaces. Whereas numerous studies have investigated the organization of these proteins 5-10 minutes after contact formation between the cell and the surface, our single-molecule imaging techniques enable us to study these processes immediately after contact establishment. Thus our experiments provide new insights into the molecular rearrangements that may underpin the initial events in T-cell activation.

610-Pos Board B379

Investigating the Dynamic Behavior of TCR Microclusters by a Gold Nanoparticle Array

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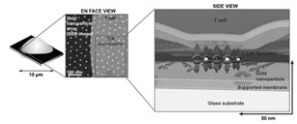
T cell receptor (TCR) microclusters play an important role in early signaling in T cells, and their properties and dynamic behavior are the active target of research. TCR microclusters are, however, difficult to study because their size is too small for conventional fluorescence microscopy and too large for physicochemical methods that depend on proximity effect such as FRET. We developed a new platform for probing and manipulating TCR microclusters by mechanical means. A two-dimensional array of gold nanoparticles is embedded on the glass substrate. A supported membrane, which acts as a surrogate cell membrane that has laterally fluid ligand molecules for cells, was deposited on a glass substrate with a regular array of gold nanoparticles. The supported membrane interacted with T cells and the behavior of TCR microclusters on the T cell surface was effectively altered by the gold nanoparticles. This platform offers many opportunities for studying this poorly understood size regime of protein assemblies.



611-Pos Board B380

SNAP-tag Fusion Proteins as a Platform for Studying T Cell Costimulation

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The costimulatory molecules CD80 and CD86 regulate T cell signaling through their interactions with CD28 on the T cell surface. Reports indicate that costimulation increases T cell activation and lowers the TCR triggering threshold. The regulatory role of these proteins is implicated in autoimmune disease and has been exploited in clinical therapies. Despite the biomedical importance of this process, the physical mechanism by which CD28 modulates T cell signaling is unknown. We have developed a platform to study various aspects of T cell costimulation using SNAP-tag fusions of CD80 and CD86. This approach provides a means for selective functionalization of the ligands through the SNAP-tag domain. The costimulatory molecules are displayed on a supported lipid bilayer (SLB) mimicking the antigen-presenting cell. By labeling each SNAP-tag with a single fluorophore, we can characterize protein mobility and dimerization via fluorescence correlation spectroscopy and photon counting. The SLB can also be chemically functionalized with peptide-MHC and ICAM-1 to trigger the activation of primary T cells interacting with the artificial membrane. Using this live T cell-supported membrane system and high-resolution imaging, we can quantify the effects of CD28- mediated costimulation on early T cell signaling.

612-Pos Board B381

The Effect of Costimulation on T Cell Receptor Dynamics

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T cells are able to robustly discern extremely low numbers of agonist peptide amidst a myriad of background peptides. Costimulation of CD28 on the T cell by CD80 on the antigen presenting cell is known to enhance the sensitivity of this recognition process, but the physical mechanisms by which these two pathways interact remain unresolved. We quantitatively characterized the T cell dose response to varying surface densities of both agonist peptide and the costimulatory ligand CD80. Using time-lapsed TIRF and confocal microscopy, the colocalization and internalization of TCR and CD80 was tracked over the standard calcium response time. TCR and CD80 spatiotemporal dynamics varied between agonist surface concentrations in a non-linear manner. Implications of these results will be discussed.

613-Pos Board B382

H-Ras Membrane Orientation Affects Galectin-1 Dependent Nanoclustering

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Members of Ras family of proteins are central regulators of crucial cellular processes. Their deregulation is associated with severe diseases, such as cancer and neuro-cardio-facio-cutaneous syndromes. Ras isoforms are very similar in structure, but at least partially show distinct biological functions. The mechanisms by which they realize different signaling outcomes are still not fully understood.

Ras proteins are membrane anchored and in the membrane they show lateral segregation into dynamic proteo-lipid domains, called nanoclusters. Nanocluster stability of H-ras is increased by galectin-1 (gal1), which can therefore be regarded as prototypic nanoclustering scaffold. We recently provided evidence that Ras adopts guanine nucleotide dependent and isoform specific orientation states on the membrane. These states are guided by a new switch III region and are stabilized by the amphipathic helix α4 and the C- terminal HyperVariable Region (HVR). Mutations on helix α4 and the HVR (orientation-mutants) of H-Ras lead to systematically modulated interactions of H-ras with the Ras binding domains of the effectors C-Raf (C-Raf-RBD) and PI3Kα in BHK cells and different ppERK signaling and PC12 differentiation outcomes. Interestingly, also gal1 differentially interacts with these H-Ras mutants.

These latter results indicated that the Ras-orientation mechanism is also relevant for its gal1-dependent nanoclustering.

In order to address, whether differential recruitment of the effectors in cells follows gal1-dependent nanoclustering, we are using sensitive cellular fluorescence microscopy techniques: FRAP to monitor immobilization of H-Ras orientation mutants due to different gal1 level, STED-FCS to compare nanoscale diffusion and FLIM-FRET to analyze the molecular complex of Ras and above mentioned binding partners.

Our current data show that different H-Ras orientation mutants have different abilities to form gal1-dependent nanoclusters, which then determines effector recruitment rates.

614-Pos Board B383

Ras Proteins, Lipid Domains and Palmitoylation: Modelling the Complex Interactions between Ras Proteins and Cell Membranes

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The Ras proteins have been repeatedly identified as key components in a wide range of cell signalling networks. The classical Ras proteins all have at least one C-terminal lipid anchor which is essential for both their membrane localisation within the cell and their biological activity. Coarse-grained molecular dynamics simulations have, over the past few years, been established as a reliable technique for examining protein-lipid interactions, both for integral and more recently for peripheral membrane proteins. We have used this technique to examine how Ras proteins bind to a model phase-separated membrane containing cholesterol. In particular, we wish to explore how Ras proteins may cluster and what effect they have on the nature and size of lipid domains. Each of the classical Ras proteins has a farnesyl lipid anchor, but HRas and NRas may also be (reversibly) palmitoylated. We have therefore examined the effect of removing one (or in the case of HRas, both) palmitoyl tails upon their interactions with membranes.

615-Pos Board B384

Functional Dynamics and Allosteric Regulation of Ras Activation by SOS

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Activation of membrane-tethered H-Ras by the exchange factor Son of Sevenless (SOS) is an important hub for signal transduction. SOS activates Ras by catalyzing the exchange of Ras-bound GDP with GTP, triggering subsequent downstream signaling in e.g. the MAP Kinase cascade. We employ supported lipid membrane arrays displaying fluorescent nucleotide-loaded H-Ras to monitor the specific activity of individual SOS molecules. In addition to